

## COPPER, ZINC, AND IRON STATUS OF FEMALE SWIMMERS

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### ABSTRACT

Twelve female swimmers underwent assessment of nutritional status at the start and end of a competitive season to determine the influence of physical training on trace element nutriture. Fat free mass increased ( $p < 0.01$ ) and fat mass decreased ( $p < 0.01$ ). Mean daily energy intake increased ( $p < 0.05$ ) because of increased carbohydrate consumption ( $p < 0.05$ ). Dietary copper, zinc, and iron did not change. Hematocrit and hemoglobin decreased ( $p < 0.05$ ) slightly, but plasma copper, ceruloplasmin, ferritin, iron, and zinc were unchanged. Red blood cell superoxide dismutase activity increased ( $p < 0.05$ ). These findings indicate that trace element nutriture is not adversely affected by physical training when dietary intakes are adequate, and that increases in superoxide dismutase activity are a functional adaptation of copper metabolism to aerobic training.

Key Words: zinc, copper, iron, human, body composition, physical activity

### INTRODUCTION

The role of nutrition in the development and the maintenance of physical performance is a continual point of interest among recreational and competitive athletes and physical fitness advocates. These individuals seek advice on nutritional practices from varied sources: peers, coaches, health professionals, and the popular press. Unfortunately, there is a paucity of authoritative information on the nutritional demands of physical training.

Studies of athletes participating in intensive physical training usually describe changes in macronutrient intakes (1-3). Information is limited on micronutrient intakes (4-6), or blood biochemical indices of nutritional status

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(7-9). Furthermore, there are no reports integrating data on trace element intakes and blood biochemical determinations of trace element nutriture of competitive athletes.

This study examined the influence of intensive swim training on copper, iron, and zinc status of young women. These athletes were studied because swimmers spend considerable periods of time training on land and in the water, and because women tend to be at a greater risk of developing some nutritional disturbances than men.

#### MATERIALS AND METHODS

All (n = 21) of the members of the University of North Dakota women's varsity swim team were studied before the start of formal preseason training and at the end of the competitive season. This investigation was approved by the Human Study Review Committees of the United States Department of Agriculture, Agricultural Research Service and the University of North Dakota. Each volunteer gave written informed consent before participating in this study.

Nutritional status was assessed by determining body composition by hydrodensitometry using the equipment and procedures described by Akers and Buskirk (10) with the modification that the strain gauges are mounted under water in the tank. Residual lung volume was measured simultaneously with the underwater weighing by an open-circuit technique for nitrogen washout of the lungs (11). Percent body fat was calculated from body density measurements according to the equation of Brozek et al (12).

Nutrient intakes were estimated from self-reported seven-day dietary records obtained from the participants who consumed self-selected diets. The diet records were reviewed at the end of each seven-day period for completeness of food descriptions and amount of food consumed. Questions were asked to identify food items that may have been overlooked. The dietary records were coded for computer calculations by trained clerical staff and checked by a home economist. The nutrient intakes were calculated by GRAND, a computerized dietary data analysis system based on USDA food composition data (13, 14) and trace element data from other published sources (15-17).

Fasting venous blood samples were obtained by phlebotomy using precautions to avoid trace element contamination. Hematology was determined using automated instrumentation (Coulter Counter<sup>1</sup>; Hialeah, FL). Serum ferritin analyses were done using a commercial radioimmunoassay kit (Clinical Assay; Cambridge, MA). Serum iron and total iron-binding capacity were measured using atomic absorption spectrophotometry (AAS; 18). Transferrin was determined using radial immunodiffusion (RID; 19).

Plasma zinc was measured by flame AAS after dilution with 2% hydrochloric acid and compared to standards containing 5% glycerol (20). Plasma copper and iron were determined and compared to standards containing 2% hydrochloric acid.

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Ceruloplasmin in the plasma was assayed both enzymatically as *p*-phenylenediamine oxidase (21) and by RID (19). Superoxide dismutase (E.C.1.15.1.1), a copper-dependent enzyme, was assayed in red blood cells using the method described by Winterbourne (22).

Values are reported as mean  $\pm$  SEM. Comparison between pre- and post-season measures of nutritional status were done using the paired t-test (23).

### RESULTS

After reviewing the diet histories, it was determined that six swimmers used oral contraceptive agents and five regularly consumed nutritional supplements; two swimmers used both. Because these preparations can influence biochemical indices of trace element status (24, 25) data from the nine women who reported using these products were excluded. Therefore, only data from 12 swimmers were included in the analysis.

It is noteworthy that each of the 12 participating swimmers secured first, second, or third place in either an individual or a relay event at the 1986 North Central Conference Women's Swimming Meet that was won by the University of North Dakota. In addition, six of the swimmers were awarded All American status at the 1986 NCAA Division II Women's National Championship at which their team captured sixth place.

Important compositional changes occurred after training. Although body mass did not change (Table 1), fat free mass increased ( $p < 0.01$ ) and fat mass decreased ( $p < 0.01$ ).

TABLE 1  
Physical Characteristics (mean  $\pm$  SEM)

Season	Stature cm	Body Mass kg	Fat-free Mass kg	Fat Mass kg
Start	168.4 $\pm$ 2.5	62.1 $\pm$ 1.7	48.7 $\pm$ 1.3	13.4 $\pm$ 0.4
End	168.4 $\pm$ 2.4	62.7 $\pm$ 1.9	51.1 $\pm$ 1.3*	11.5 $\pm$ 0.3*

\* $p < 0.01$ ,  $n = 12$

Analysis of dietary records indicated that swim training was associated with a 12% increase ( $p < 0.05$ ) in mean daily energy intake (Table 2). Whereas protein and fat consumption were unchanged, carbohydrate intake increased ( $p < 0.05$ ) by about 60 g/d.

TABLE 2  
Macronutrient Intakes (mean  $\pm$  SEM)

Season	Energy kcal/d	Protein g/d	Carbohydrate g/d	Fat g/d
Start	2030 $\pm$ 96	61 $\pm$ 6	271 $\pm$ 20	77 $\pm$ 13
End	2269 $\pm$ 104*	60 $\pm$ 4	334 $\pm$ 17*	76 $\pm$ 8

\* $p < 0.05$ ,  $n = 12$

Although daily trace element intakes did not change significantly during physical training (Table 3), there was a trend to decreased micronutrient consumption.

TABLE 3  
Estimated Trace Element Intakes (mean  $\pm$  SEM)

Season	Copper mg/d	Iron mg/d	Zinc mg/d
Start	1.1 $\pm$ 0.1	13.2 $\pm$ 0.8	10.2 $\pm$ 0.7
End	1.0 $\pm$ 0.1	12.1 $\pm$ 1.0	9.7 $\pm$ 0.8
Guideline	1.3*	12 <sup>+</sup>	9.9 <sup>+</sup>

\*Defined as 67% Estimated Safe and Adequate Intake (26)

<sup>+</sup>Defined as 67% Recommended Dietary intake (26)

To estimate the adequacy of the swimmers' trace element intake, the average calculated intake was related to a general guideline representing 67% of the level recommended for the American public. In these terms, it appears that intakes of iron and zinc are consistent with this guideline (Table 3). Copper intake appears to be low based upon the Estimated Safe and Adequate Intake level proposed (26).

Some blood biochemical indices of nutritional status changed (Table 4). Small reductions ( $p < 0.05$ ) in hematocrit and hemoglobin, and an increase in total iron-binding capacity were observed. Ferritin, a measure of body iron stores, was unchanged. All of the measured hematological and ferritin values were within the reference range for healthy women.

TABLE 4  
Blood Biochemical Indices of Iron Status (mean  $\pm$  SEM)

Season	Hematocrit %	Hemoglobin g/dl	Ferritin ng/ml	Total Iron Binding Capacity mg/dl	Transferrin %
Start	40.8 $\pm$ 0.4	13.9 $\pm$ 0.2	18 $\pm$ 5	301 $\pm$ 15	36 $\pm$ 4
End	39.4 $\pm$ 0.7*	13.2 $\pm$ 0.2*	25 $\pm$ 4	339 $\pm$ 17*	30 $\pm$ 5
Reference <sup>+</sup>	37 - 47	12 - 15	12 - 50	258 - 418	20 - 50

\* $p < 0.05$

<sup>+</sup>Range of normal values for Grand Forks women

The measured plasma concentrations of copper and enzymatic (ENZ) and immunoreactive (RID) ceruloplasmins were unchanged with training (Table 5). Similarly, plasma iron (102  $\pm$  6 vs 94  $\pm$  10  $\mu$ g/dl) and zinc (81  $\pm$  2 vs 77  $\pm$  3  $\mu$ g/dl) did not change. There was a slight tendency for ceruloplasmin, iron, and zinc to decrease. The observed plasma concentrations of trace elements were within the range of normal values. Erythrocyte superoxide dismutase activity increased significantly after swim training.

TABLE 5  
Blood Biochemical Indices of Copper Status (mean  $\pm$  SEM)

Season	Plasma Copper $\mu\text{g/dl}$	Ceruloplasmin ENZ* mg/L	Ceruloplasmin RID* mg/L	Superoxide Dismutase units/g Hgb
Start	95 $\pm$ 11	419 $\pm$ 37	283 $\pm$ 20	4193 $\pm$ 170
End	94 $\pm$ 10	397 $\pm$ 38	258 $\pm$ 29	5139 $\pm$ 183 <sup>+</sup>
Reference**	55 - 160	220 - 430	180 - 380	1850 - 5250

\*Plasma ceruloplasmin concentration was determined enzymatically (ENZ) and by radial immunodiffusion (RID)

<sup>+</sup>p < 0.004

\*\*Range of normal values for Grand Forks women

### DISCUSSION

The results of the present study demonstrate that the measured indices of copper and zinc status were not reduced in the swimmers after five months of training. This observation conflicts with the results of some previous studies that implied that physical training alters elemental nutriture.

#### Copper.

Dowdy and Burt (8) followed competitive male swimmers through a six-month training period and observed that ceruloplasmin decreased from 37 IU during the first month of training to 25 IU in the second month, and it remained at that level for the remainder of the study. Serum copper concentrations averaged 64  $\mu\text{g/dl}$  during the first half of the study, and it dropped to a mean of 50  $\mu\text{g/dl}$  over the final three months.

In contrast to these findings, several cross-sectional studies (9, 27) found that male athletes exhibited significantly higher serum (116 vs 93  $\mu\text{g/dl}$ ) or plasma (90 vs 81  $\mu\text{g/dl}$ ) copper concentrations than age-matched sedentary men. Another report (28) stated no difference in serum copper concentrations between male runners and controls (96 and 93  $\mu\text{g/dl}$ , respectively). These observations are consistent with the findings of the present study in which plasma copper and ENZ and RID ceruloplasmin did not change during training.

In addition to monitoring circulating concentrations of copper, we investigated changes in an enzyme whose activity is dependent upon the presence of copper. Superoxide dismutase is a copper and zinc containing enzyme, whose activity is dependent upon copper and whose conformation or structure requires zinc (29). Erythrocyte superoxide dismutase activity increased significantly after swim training.

Free radicals such as the superoxide anion are produced as a result of normal cellular oxidative metabolism. Increased amounts of superoxide radicals are associated with lipid peroxidation and the accumulation of metabolites that can lead to cell injury or death (30). An important mechanism that controls damage caused by oxygen radicals is superoxide dismutase (29). Fatiguing exercise has been shown to increase free radicals in rodent muscle and liver (31). Furthermore, superoxide dismutase activity from human skeletal muscle

was significantly correlated with peak oxygen uptake (32). Thus, increases in superoxide dismutase activity may be an enzymatic adaptation to aerobic training that protects against oxidative damage to tissues.

It is noteworthy that despite a copper intake of 1.0-1.1 mg/d, we observed a significant increase in erythrocyte superoxide dismutase activity. This indicates a training-specific biochemical adaptation in copper metabolism that may be important in reducing free radical damage to muscle during intensive training (32). Also, this may be an example of copper redistribution as a consequence of chronic training.

#### Zinc.

Hypozincemia also has been reported among some groups of athletes. Dressendorfer and Sockolov (28) found reduced ( $p < 0.05$ ) concentrations of serum zinc in male long-distance runners relative to sedentary men (76 vs 94  $\mu\text{g/dl}$ ). They also observed that serum zinc was inversely related to training distance with average serum zinc concentrations of 81 and 67  $\mu\text{g/dl}$  for runners training 6-12 and 40-84 miles/wk, respectively. Haralambie (33) screened 160 athletes (57 women and 103 men) and noted that 43% of the women and 23% of the men had serum zinc concentrations less than 75  $\mu\text{g/dl}$ , the designated limit of their range of normal values. Previous results from our laboratory (9) found similar plasma zinc values among male athletes and nonathletes (87 and 87  $\mu\text{g/dl}$ , respectively). This latter finding is consistent with the present data indicating no change in plasma zinc after training.

#### Iron.

The iron status of an athlete is recognized as influencing work performance because iron deficiency anemia impairs aerobic capacity (34). However, the prevalence of iron deficiency anemia is not consistently greater among athletes. Some investigators (35, 36) conclude that athletes do not exhibit a greater tendency toward anemia than untrained controls. It also appears that female athletes do not have a greater tendency to manifest iron-deficiency than men (37). However, some indications of developing iron deficiency among athletes have been reported.

Haymes (38) concluded that 22-25% of female athletes and less than 10% of male athletes have low plasma iron concentrations even though many had hemoglobin concentrations exceeding those indicative of anemia. Similarly, Kilbom (39) found a 25% decrease in serum iron in women after a two month exercise program. Deuster et al (40) reported that in a group of 51 highly trained female runners, 35% had ferritin concentrations less than 12 ng/ml which is indicative of depleted body iron stores (41).

In the present study, although hemoglobin and hematocrit decreased slightly, none of the individual values were indicative of anemia. Total iron binding capacity increased and plasma iron declined suggesting a marginal alteration of iron transport. Serum ferritin was unchanged. Thus, iron status was not markedly impaired by the swim training.

Small, though significant decreases in hematocrit and hemoglobin were observed as iron consumption declined slightly from 13.2 to 12.1 mg/d during training. The physiological importance of these reductions in hematology is unknown because none of the swimmers became anemic.

One physiological explanation for the modest decline in hematocrit and hemoglobin is the expansion of plasma volume associated with chronic aerobic training. Estimates of plasma volume expansion range from 7-20% (43), and are consistent with the observed dilutions in plasma constituents seen in this study.

#### Dietary Intake.

Interpretation of blood biochemical indices of trace element status during physical training requires information about the intake of these nutrients. Among the swimmers, there were no significant changes in plasma copper, ceruloplasmin, iron, or zinc with training. Similarly, consumption of iron and zinc did not change, and were within acceptable ranges of intake. The only exception was copper, with an intake of 1.0-1.1 mg/d. This copper intake is less than the suggested level of 2 mg/d (26), but is similar to the reported intakes of other groups in the United States (42).

#### Body Composition.

In addition to assessing trace element status, body composition was determined. Fat free mass increased and fat mass decreased after swim training. These structural changes have been shown previously to be associated with improved swimming performance in women (44).

### CONCLUSIONS

Our data indicate that intensive swim training for competition is associated with favorable changes in body composition and does not impair trace element nutriture when dietary intake of these nutrients are adequate. The slightly lower circulating levels of trace elements after training are probably the result of plasma volume expansion. These findings indicate that physical training per se does not produce adverse effects on trace mineral nutritional status.

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